

# Is Behçet's disease a 'class 1-opathy'? The role of HLA-B\*51 in the pathogenesis of Behçet's disease

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## Summary

The association between carriage of the human leucocyte antigen (HLA)-B\*51 allele and development of Behçet's disease (BD) has been known since the early 1970s, but the exact mechanisms responsible for its role in pathogenesis remain much-debated. In an effort to explain the disease process, it has been suggested that BD constitutes one of a newly termed group of diseases, the 'MHC-I-opathies'. Other MHC-I-opathies include ankylosing spondylitis and HLA-B\*27-associated spondyloarthropathies and HLA-C\*0602-associated skin psoriasis. Recent work analysing the peptidome of HLA-B\*51 suggests that altered peptide presentation by HLA-B\*51 is vital to the disease process. In this review, we argue that immune receptor interactions with HLA-B\*51 or the HLA-B\*51-peptide complex could lead to development of inflammation in BD. The evidence for CD8<sup>+</sup> T cell involvement is weak, and based on emerging studies it seems more likely that natural killer (NK) or other cell interactions, perhaps mediated by leucocyte immunoglobulin-like receptor (LILR) or killer immunoglobulin-like receptor (KIR) receptors, are culpable in pathogenesis. HLA misfolding leading directly to inflammation is another hypothesis for BD pathogenesis that deserves greater investigation. Ultimately, greater understanding of HLA-B\*51's unique role in BD will probably lead to improved development of therapeutic strategies.

**Keywords:** antigen presentation/processing, autoimmunity, autoinflammatory disease

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## Introduction

Behçet's disease (BD) is a rare chronic multi-system inflammatory disorder characterized by recurrent relapsing oral and genital ulcers, ocular involvement, skin lesions, arthritis and vasculitis [1]. BD typically develops clinically in the third and fourth decades of age, with earlier age of onset being correlated with higher severity and mortality [2].

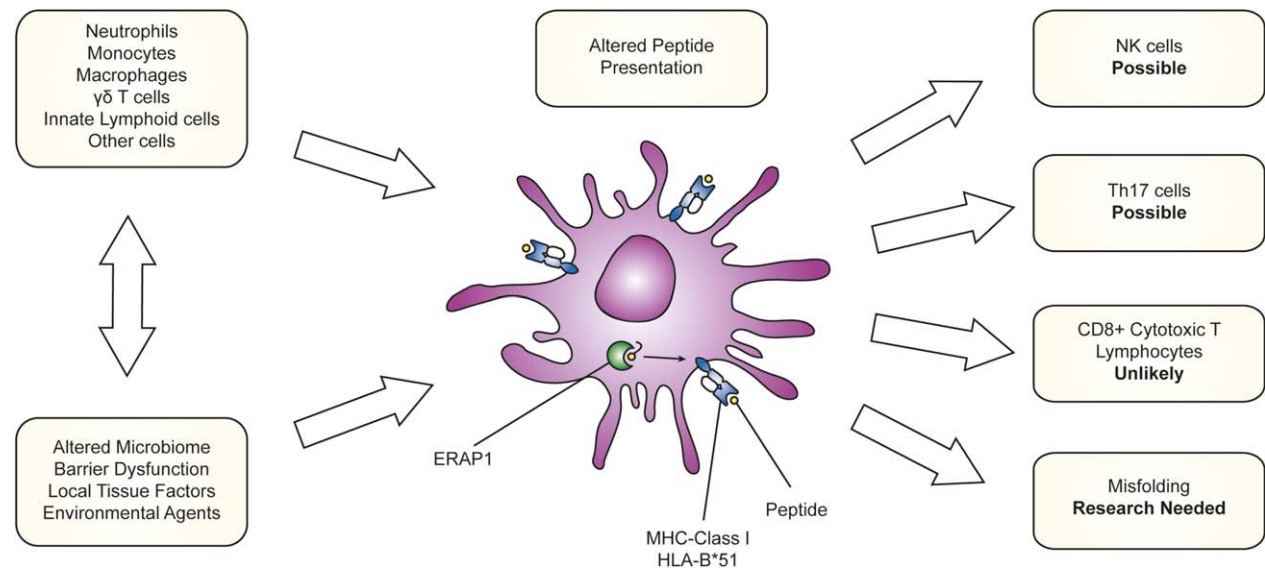
Human leucocyte antigens (HLAs) are proteins encoded by genes that make up the human major histocompatibility complex (MHC). Three different genes, designated HLA-A, -B and -C, encode MHC class I proteins expressed on the surface of almost every nucleated cell in the body, and are a vital marker of self. These MHC class I proteins present peptides from both endogenous and exogenous proteins found within the cell on the cell surface for immune cells to survey. HLA-B\*51 is one of many MHC class I variant alleles.

For many years, the human leucocyte antigen (HLA) B\*51 has been linked to the development of Behçet's disease

(BD). This review will dissect the possible mechanisms explaining how HLA-B\*51 allele carriage leads to BD, concluding that its probably altered MHC class I peptide presentation plays a role. Despite being the most intuitive possibility to explain pathogenesis, the evidence for activation of CD8<sup>+</sup> T cells by HLA-B\*51 is not compelling. Interactions of HLA-B\*51 molecules with natural killer (NK) cells or NK family receptors expressed on leucocytes could be causative to disease development, but this must be investigated further. Protein misfolding (an area of little current research) or even alternative mechanisms could be responsible for the role of HLA-B\*51 in BD pathogenesis (Fig. 1).

## Current theories of BD pathogenesis

BD is thought to lie on a spectrum between autoinflammatory and autoimmune diseases, sharing features of both [3–5] (Table 1). While the innate immune system causes



**Fig. 1.** Potential role of human leucocyte antigen (HLA)-B\*51 in Behçet's disease (BD) pathogenesis.

inflammation in autoinflammatory disorders, in autoimmune diseases the activated adaptive immune system is responsible [6]. Assessing the evidence, it seems that BD shares more common features with autoinflammatory diseases. Despite this, Yazici highlights several key differences between BD and the archetypal autoinflammatory disorders, such as familial Mediterranean fever [4]. These include a lack of associations with characteristic innate immune mutations in inflammatory and apoptotic pathways [tumour necrosis factor (TNF) receptor, pyrin, caspase recruitment domain (CARD)/nucleotide-binding oligomerization domain (NOD) genes]; a unique disease course featuring extensive vasculitis; and the disease severity regressing with older age.

Because of this, McGonagle has proposed that BD be defined as an 'MHC-I-opathy' alongside spondyloarthropathies such as ankylosing spondylitis (AS), reactive arthritis, psoriatic arthritis (PsA) and arthritis associated with inflammatory bowel disease – all of which share an association with MHC class I alleles [7]. AS has long been known to have a very strong association with HLA-B\*27 [8]. These diseases all share common features, including HLA class I associations, evidence of enhanced helper type 17 (Th17) immune responses and areas of prominent neutrophilic

inflammation. Additionally, barrier dysfunction and aberrant innate immune reactions at sites of stress may contribute.

Based on these criteria, BD appears to fit most closely into this new MHC-I-opathy category. McGonagle suggests a key role for CD8<sup>+</sup> T cells in activating neutrophils and driving inflammation directly as being a common component of these diseases [7]. We believe that the current evidence for CD8<sup>+</sup> T cell involvement is weak, and propose that 'non-classical' activities and interactions of HLA-B\*51 are at least as likely to be pathogenic in disease. A clearer understanding of all the functions of HLA-B\*51 is thus needed in order to improve development of potential therapeutics based on the rational targeting of responsible mechanisms.

### Genetic links between HLA-B\*51 and BD

The link between carriage of HLA-B\*51 and BD development was first highlighted in 1973, with a higher frequency of what was then known as HL-A5 observed in patients ( $n = 21$ ) compared to controls ( $n = 78$ ) [9]. Many studies have been performed since then, creating a large body of evidence linking HLA-B\*51 with susceptibility for BD. A

**Table 1.** Features of autoinflammatory and autoimmune diseases shared by Behçet's disease (BD)

Autoinflammatory features	Autoimmune features
Increased activity of neutrophils	MHC class I association (HLA-B*51)
Elevated levels interleukin-1β	Some symptoms can be relieved with T cell suppressing agents
Recurrent episodes of remission and exacerbation	Tenuous evidence of autoreactive T cells
Enhanced inflammatory response and overexpression of inflammatory cytokines	Arthritis and skin involvement – similar to systemic lupus erythematosus (SLE)
No specific autoantibodies	

MHC = major histocompatibility complex; HLA = human leucocyte antigen.

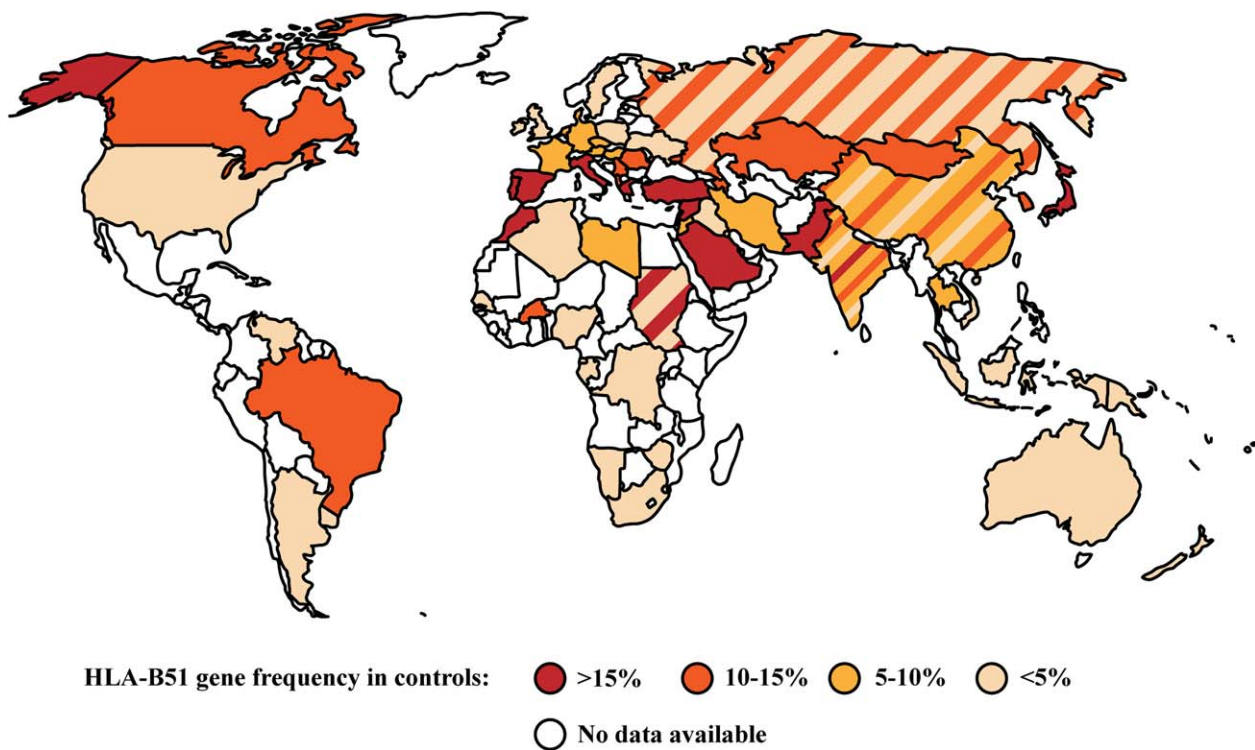


Fig. 2. Approximate global prevalence of human leucocyte antigen (HLA)-B\*51. Data taken from Verity [15] and based on studies performed in each country. Striped colour coding indicates more than one reported study of different prevalence rates.

meta-analysis of 80 studies performed in a variety of different populations suggests that carriage of HLA-B\*51 or HLA-B5 (the group of antigens of which B\*51 is a subcategory) confers an odds ratio (OR) of 5.78 for developing BD and 5.90 when considering only HLA-B\*51, without a significant modifying effect of ethnicity [10]. The possibility that this relationship represents linkage disequilibrium (LD) with a nearby and truly causal BD gene is unlikely, for a number of reasons. First, the likelihood that a genetic association takes account of confounding diminishes with the strength of the association, and an unbiased OR of greater than 3–5 is deemed to be strongly in favour of a causal variant [11]. Secondly, nearby genetic variants that are in strong LD with HLA-B\*51 have been shown to be associated much less strongly with BD than HLA-B\*51 itself. A recently proposed nearby causative single nucleotide polymorphism (SNP) (rs116799036) [12] was shown by Ombrello *et al.* using conditional analysis to be associated only weakly with BD and HLA-B\*51 continued to have a significant association even after conditioning for rs116799036 [13]. Thirdly, the association has been found in multiple ethnic groups. Finally, HLA-B\*51 is itself a plausible contributor to disease, with MHC class I molecules linked to a variety of diseases [7].

Further evidence for the link between HLA-B\*51 and BD comes via its geographical epidemiology. BD has been known as the 'Silk Road disease' [14], reflecting its

prevalence along the ancient trading route from East Asia to the Middle East and the Mediterranean, where there is both a high prevalence of HLA-B\*51 in the population (Fig. 2) and a higher prevalence of BD [15]. For example, in the United Kingdom the prevalence of HLA-B\*51 in controls is 3–8% and BD prevalence 0.27–0.5 per 10<sup>5</sup> of population, compared to 24% and 90–100 per 10<sup>5</sup>, respectively, in Turkey. Verity *et al.* [15] used a Spearman's rank test to show a close correlation between latitude (a good marker of HLA-B\*51 prevalence) and BD prevalence ( $P < 0.05$ ). However, certain Amerindian populations in Alaska and Canada have been found to have a high HLA-B\*51 prevalence but, conversely, zero reports of BD [16]. This may be due to the absence of an external risk factor, but it is probably more likely that local physicians are less familiar with the disease, so it is under-reported, and the total expected cases would be very low in a population of only ca. 140 000 based on the BD prevalence in other countries and ethnic groups.

The evidence of a heritable genetic risk for BD comes from familial studies. This was quantified for a Turkish population by Gül *et al.* [17], showing a sibling recurrence risk ratio ( $\lambda_s$ ) of between 11.4 and 52.5 in different populations within Turkey. Hence, although the inheritance of BD does not follow a simple Mendelian pattern, there appears to be a genetic link with a  $\lambda_s$  higher than some other genetically complex diseases, such as rheumatoid arthritis

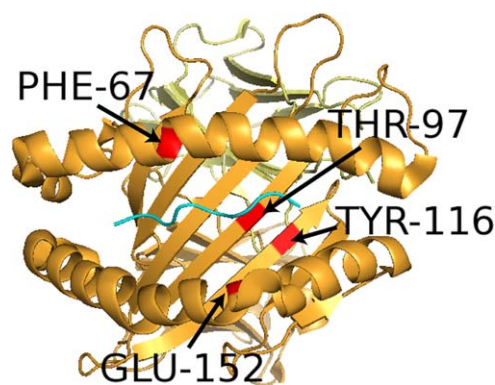
( $\lambda_s = 6$ ) [18] and type I diabetes mellitus ( $\lambda_s = 15$ ) [19]. Additionally, a retrospective study in Sweden found increased previous family history in patients with early-onset 'paediatric BD' (12.3%) compared with adult patients (2.2%) [20].

These three examples of evidence confirm the significant genetic contribution to the development of BD, and that HLA-B\*51 has some causal role. This link is not as strong as other MHC-I-opathies such as AS, which has an OR of 171, and 94% of patients carrying the risk allele [21] compared to only 59% in BD [22]. It is therefore more likely that HLA-B\*51 plays only one role in a complex polygenic disease pathogenesis. It is the most unique aspect of BD, however, and thus could be the key to understanding the disease.

### Pathogenesis via altered peptide presentation

The identity of HLA-B\*51 as a class I MHC molecule means that abnormal presentation of endogenous or pathogenic peptides is an attractive and intuitive hypothesis for its role in BD pathogenesis. Certain amino acid residues within the antigen-binding groove of the HLA-B protein influence the risk of developing BD. Polymorphisms at positions 97, 116, 152 and 67 of the HLA-B protein influence the OR for BD significantly and independently [13] (Fig. 3). These four residues contact six of the nine amino acids of the presented peptide antigen and act to define the sizes and shapes of peptides accommodated in the MHC I groove. Additionally, residues 67 and 116 are hypothesized to be critical anchor residues, defining the peptide specificity of MHC I antigen binding via interactions with peptide positions P2 and P $\Omega$  (P9), respectively [23,24]. Moreover, HLA-B residue 67 is one of two amino acids that differ between HLA-B\*51 and HLA-B\*52, a near-identical HLA-B protein which confers no significant effect on BD risk [13]. This seems strong evidence to suggest that altered peptide presentation utilizing these amino acids may explain, at least in part, the link between HLA-B\*51 and disease pathogenesis.

A recent genomewide association study showed a link between BD risk and polymorphisms in endoplasmic reticulum aminopeptidase 1 (ERAP1) [26]. ERAP1 functions in the endoplasmic reticulum (ER) to trim the aminoterminal residues of precursor peptides to an optimal length for loading onto class I MHC molecules [27,28]. Two SNPs in ERAP1, encoding p.Asp575Asn and p.Arg725Gln, conferred a risk for BD preferentially in HLA-B\*51-positive individuals from a large Turkish sample. Homozygosity for p.Arg725Gln was associated with an OR for BD of 3.78 [95% confidence interval (CI) = 1.94–7.35] in HLA-B\*51-positive individuals compared with 1.48 (95% CI = 0.78–2.80) in HLA-B\*51-negative individuals, indicating that the ERAP1 variant contributes to disease susceptibility via interaction with the HLA-B\*51 protein [26]. Indeed, this



**Fig. 3.** Structure of the major histocompatibility complex (MHC) class I molecule human leucocyte antigen (HLA)-B\*51, showing Behçet's disease (BD)-associated amino acid positions and bound peptide. Pale blue indicates complexed peptide (HIV immunodominant epitope KM2). Image of Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank ID: 1E28 [25] drawn using PyMOL (the PyMOL Molecular Graphics System, version 1.8 Schrödinger, LLC). Red indicates amino acids with genome-wide significant association ( $P < 5 \times 10^{-8}$ ) with BD [13]. PHE = phenylalanine; THR = threonine; TYR = tyrosine; GLU = glutamic acid.

study showed ERAP-1 homozygosity was found in a higher percentage of patients than controls, but this was still in only 7.62% compared to 1.92%. Further light was shed on this possible pathogenic mechanism when the peptidome of HLA-B\*51:01 (a subtype of HLA-B\*51) was characterized extensively (> 1600 peptides). Two major peptide subsets were identified, with either Pro or Ala at position 2 (total frequency 89.3%, Pro : Ala ratio 1.9) and, importantly, both subsets differed drastically in the susceptibility of their position 1 residues to ERAP-1 [29]. These susceptibilities were based on results of experiments by Hearn *et al.* [30] looking at ERAP1 degradation of peptides with different N-terminal amino acids.

The risk ERAP-1 allotype with p.Asp575Asn and p.Arg725Gln SNPs confers lower enzymatic activity and alters the balance between these two peptide subsets, with lower-affinity Ala2 peptides being presented more on HLA-B\*51 [29]. In addition to these findings, AS and psoriasis are also associated with ERAP-1 in epistasis with their susceptibility MHC class I alleles and may share similar pathogenesis mechanisms [31,32]. Together, these findings provide strong evidence that altered antigen presentation by HLA-B\*51 plays a role in the pathogenesis of BD.

Moreover, in a recent study, an ERAP1 protein allotype called Hap10 was associated with BD risk in HLA-B\*51 carriers following the recessive model [33]. This haplotype carried five non-ancestral alleles, including p.Asp575Asn and p.Arg725Gln, and it was found previously to have lower peptide-trimming activity [29,34]. HLA-B\*51 carriers homozygous for the haplotype had an 11-fold

increased disease odds compared with individuals who had none of the genetic risk factors. We think it likely that an individual's ERAP allotype alters the peptides available for binding to HLA-B\*51, thus influencing the peptidome and contributing to increased disease risk.

### Microbiome involvement

An idea currently being explored in BD pathogenesis is that the gut or other microbiomes such as skin or oral sites might be involved. This is established somewhat more clearly in other diseases such as AS, psoriasis and PsA. For example, in AS there is evidence of gut microbiome dysbiosis with an abundance of the genus *Dialister* correlated with disease activity [35]. Supporting this information, Lin *et al.* [36] showed that the gut microbiota is different in HLA-B\*27 transgenic mice compared with wild-type mice, showing a correlation between possession of HLA-B\*27 and the shaping of the gut microbiome. In patients with skin psoriasis and PsA a recent study found that the diversity of the gut microbiome was lower compared to healthy controls, due to a lower abundance of several taxa [37]. This profile for PsA patients seems to be similar in patients with inflammatory bowel disease, as described previously [38–40].

In BD, few data are available as the field is only now beginning to be explored, but infectious agents such as bacteria/viruses have been suggested as instigators of dysfunction in the inflammatory response in BD patients. In a recent study by Coit *et al.* [41] of Turkish patients with BD the salivary microbiome profile was found to be less diverse than in controls by using high-throughput sequencing of the V4 region of the bacterial 16S rRNA. They found that *Haemophilus parainfluenza* was the most abundant species in BD, whereas *Alloprevotella rava* and species in the *Leptotrichia* genus were less abundant. Another study by Consonlandi *et al.* [42] showed gut microbiota dysbiosis and decreased production of butyrate in patients with BD compared to healthy controls. Finally, the oral microbial flora have also been implicated in the pathogenesis of the disease, with *Streptococcus* and streptococcal infections being the most common in the oral cavity of BD patients with unhygienic oral conditions [43].

Overall, there is thus emerging evidence supporting the involvement of the microbiome in the pathogenesis of BD. Also, as it seems to play an important role in other MHC-I-opathies such as AS, psoriasis and PsA, it is logical to speculate that it may play an important role in BD as well.

### Th17 and cytokine involvement

A number of cytokines have been implicated in BD. Th17 cells, an effector population of Th cells that produce IL-17, have also been implicated recently in BD. Thus, increased frequencies of Th17 cells as well as interferon (IFN)- $\gamma$

expressing Th17 cells have been reported in the skin lesions of patients with BD compared to healthy controls [44]. Another study demonstrated that in active BD the percentage of circulating Th17 cells and plasma interleukin (IL)-17A levels were increased [45]. Chi *et al.* [46,47] found elevated levels of IL-17A, IL-23 and IFN- $\gamma$  not only in peripheral blood but also in the aqueous fluid in the eyes of BD patients. Elevated IL-6 has also been associated with disease activity. BD patients suffering from uveitis have elevated levels of IL-12 and IFN- $\gamma$  [48]. A recent study by Talaat *et al.* [49] showed that serum levels of IL-10 in BD patients were reduced compared to healthy controls, although Ben Ahmed *et al.* [50] reported increased IL-10 (and IFN- $\gamma$ ) in patients with active BD. It has now become obvious that cytokines play a crucial role in BD, although the role of HLA-B51 in cytokine polarization is unknown.

### Lack of evidence of CD8<sup>+</sup> T cell involvement

As the classical role of class I MHC molecules is to present peptides to CD8<sup>+</sup> T cells, one might expect abnormal numbers, phenotype and/or function of CD8<sup>+</sup> T cells in BD. While immunosuppressive agents with T cell inhibitory actions such as cyclosporin A have been used in treatment, their *in-vivo* targets in BD patients' disease cannot be known for certain. Also, T cells reactive to either self- or pathogenic peptides have not been implicated reproducibly in BD [51], with the exception of a small study that found autoreactive CD8<sup>+</sup> T-cells to MHC class I chain-related gene A (MICA). MICA is a stress-inducible antigen expressed preferentially on epithelium and endothelium [52]. Instead, histopathological observations implicate alternative cell types, including neutrophils, eosinophils, macrophages and CD4<sup>+</sup> T cells in infiltrations around arterioles and in lesions [1]. Candidate T cell antigens with potential pathogenic roles in BD development include heat shock proteins (HSPs). Both HSP60 (60 kDa) and HSP65 (65 kDa) are expressed under denaturing stress conditions and found abundantly in BD ulcers, and microbial HSP variants also exist with considerable sequence homology. Cross-reactive B and T cells have been found to both HSP60 and HSP65 in some small studies, but not repeatedly [53]. However, recent studies have not focused on HSPs as being vital to pathogenesis. Therefore, despite being the intuitive explanation, there is no convincing case for HLA-B\*51 cytotoxic CD8<sup>+</sup> T cells as being the causative agent in BD lesions and ulcers.

### Possible link between HLA-B\*51 and NK cells

Abnormal peptide presentation may, instead, lead to BD pathogenesis via interactions with NK cells [54]. HLA-B\*51 is known to react with an inhibitory killer immunoglobulin-like receptor (KIR) receptor, KIR3DL, found on NK cells via its Bw4 epitope [55]. The interaction

between the two leads to regulation of NK cell activity. Two potential mechanisms have been proposed to explain the pathogenesis of BD involving NK cells by Wallace [54]: NK cells lacking appropriate inhibitory KIR may fail to recognize self-MHC and cause autologous tissue damage, or defects in the NK cell repertoire may permit persistent viral infections which results in a chronic inflammatory response in BD.

However, even though numbers of NK cells were found to be increased significantly in active BD, their cytotoxic function was similar to controls and patients with inactive disease [56]. In contrast, others have found no difference between numbers of NK cells in patients and controls [57]. Furthermore, no association was found between KIR3DL1 expression on NK or T cells in patients with BD, despite the presence of the Bw4 motif [57], and no clear genetic association with other KIR receptors and HLA-B\*51 has been demonstrated [58]. During the last decade, many novel KIR receptors have also been described. These have not yet been tested for their association with BD, making this a fertile area for future research avenues. The second hypothesis is undermined by the fact that no link has been confirmed between BD and any viral infections. Scans for proposed possible causative microbes such as herpes simplex virus, Epstein–Barr virus and cytomegalovirus lead to negative results in BD patients, regardless of whether they are in a period of symptom flare-up or disease remission [59–61].

We have proposed previously for AS that quantitative or qualitative differences in HLA class I heavy chain expression have localized proinflammatory effects through interactions with immunoreceptors. Here we suggest as a third hypothesis that a similar mechanism may operate for BD.

The implications of the work by Guasp on analysing the peptidome of HLA-B\*51 has not been discussed until now with regard to the possible BD pathogenic mechanisms. Lower-affinity peptidomes, as would be created by the risk variant of ERAP1, enhance lysis by NK cells [62]. Thus, altered peptide presentation by ERAP1 and HLA-B\*51 may interact with NK cells to lead to pathogenesis.

NK cell interactions with HLA-B\*51 remain an attractive proposition for explaining BD pathogenesis; however, further research needs to be conducted into ‘inhibitory’ NK receptors that appear to actually play a more regulatory role.

### Misfolding of HLA proteins in BD pathogenesis

Alternative pathogenic hypotheses linking HLA-B\*51 with BD might include effects on the gut microbiome or of HLA-B\*51 misfolding, both mechanisms suggested for HLA-B\*27 and AS [8]. Thus, inflammation in BD may be associated directly with the identity of HLA-B\*51 as a ‘slow-folding’ MHC molecule during peptide binding [63]. Misfolding leads to ER stress and an unfolded protein response leading to inflammation. Perhaps some combination of the availability of presented peptides and misfolded

proteins leads to BD pathogenesis. This hypothesis has not yet been investigated in BD patients and warrants greater scientific thought, given that several small investigational studies show that it may play a role in AS pathogenesis with HLA-B\*27 [8].

### Non-HLA genetic aspects of BD pathogenesis

Despite this review focusing on the role of HLA-B\*51, it must be understood in the wider multi-factorial disease process. McGonagle *et al.* [7] proposed that the initiation of BD can be attributed to local factors at sites of mechanical stress and barrier dysfunction at environmentally exposed organs. Additionally, an exaggerated innate immune response is well documented, with neutrophils, macrophages and other innate immune cells frequently present in the lesions and erythematous papules that appear spontaneously in patients [1]. Targeted deep resequencing implicates rare variants in innate immune genes such as Toll-like receptor 4 (TLR-4), Mediterranean fever gene (MEFV) and nucleotide-binding oligomerization domain-containing protein 2 (NOD2) [64] in the disease process. Levels of an array of inflammatory cytokines have also been found to be raised in BD patients [1], explaining the success in recent years of anti-cytokine biologicals as treatments for BD patients [5]. The literature documenting this exaggerated innate immune response in BD is well reviewed by Gül [5]. In addition, there may well be a role for SNPs identified by genome-wide association studies (GWAS) as being associated with BD at a genome-wide association eight level ( $P < 5 \times 10^{-8}$ ) [22,26,65–70], reviewed by Takeuchi [71]. McGonagle shows how similar factors have been found in the pathogenesis of the other HLA-linked spondyloarthropathies [7]. Interactions by HLA-B\*51 with these other aspects of the immune system are thought to underpin BD, and an adequate explanation of its role must take these factors into account.

### Conclusions

Further research is required before the link between HLA-B\*51 and BD can be explained mechanistically. However, we propose that altered peptide binding by HLA-B\*51 molecules plays a key role in disease pathogenesis through effects on its cell biology and immune function. The significant association between amino acids in the peptide binding groove with disease risk is compelling evidence for this. Additionally, the work by Guasp *et al.* analysing the HLA-B\*51 peptidome implicates susceptibility to ERAP1 in the disease process, and warrants further research in this area. Interactions with NK cells by these HLA molecules and their presented peptides also seem an attractive prospect to explain the disease process. An improved understanding of the HLA-B\*51 associations (unique to BD) may ultimately help the development of novel therapeutic approaches.

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## Disclosure

The authors declare no conflicts of interest.

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